

In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 19, lines 10-12 and replace it with the following paragraph:

Fig. 9a is a) a diagram showing the map of pTip-NH1, pTip-CH1, pTip-LNH1, pTip-LNH1, pTip-NH2, pTip-CH2, pTip-LNH2 and pTip-LCH2. The function of each domain and the map of the plasmids are shown (6x His tag disclosed as SEQ ID NO: 114);

Please delete the paragraph on page 19, lines 13-15 and replace it with the following paragraph:

Fig. 9b shows b) the DNA sequence from *TipA* gene promoter sequence of pTip-NH1, pTip-LNH1, or from *TipA-LG10* promoter sequence to the multicloning site and the transcription termination sequence of *ThcA* gene (SEQ ID NOS 115-116 and 130);

Please delete the paragraph on page 19, lines 16-18 and replace it with the following paragraph:

Fig. 9c shows c) the DNA sequence from *TipA* gene promoter sequence of pTip-CH1, pTip-LCH1, or from *TipA-LG10* promoter sequence to the multicloning site and the transcription termination sequence of *ThcA* gene (SEQ ID NOS 117-118 and 130);

Please delete the paragraph on page 19, lines 19-21 and replace it with the following paragraph:

Fig. 9d shows d) the DNA sequence from *TipA* gene promoter sequence of pTip-NH2, pTip-LNH2, or from *TipA-LG10* promoter sequence to the multicloning site and the transcription termination sequence of *ThcA* gene (SEQ ID NOS 119-120 and 131);

Please delete the paragraph on page 19, lines 22-24 and replace it with the following paragraph:

Fig. 9e shows e) the DNA sequence from *TipA* gene promoter sequence of pTip-CH2, pTip-LCH2, or from *TipA-LG10* promoter sequence to the multicloning site and the transcription termination sequence of *ThcA* gene (SEQ ID NOS 121-122 and 131);

Please delete the paragraph on page 19, lines 25-26 and replace it with the following paragraph:

Fig. 10 is a diagram showing the map of pTip-CH1.1, pTip-LCH1.1, pTip-CH2.1 and pTip-LCH2.1 (SEQ ID NOS 123-126, respectively, in order of appearance);

Please delete the paragraph on page 20, lines 14-15 and replace it with the following paragraph:

Fig. 17 is a photograph in which the result of purification 2 of the foreign protein using the inducible expression vector (6x His tag disclosed as SEQ ID NO: 114);

Please delete the paragraph on page 20, lines 22-23 and replace it with the following paragraph:

Fig. 21 is a figure showing expression of the foreign protein using *Rhodococcus erythropolis* and *E. coli* as a host (6x His tag disclosed as SEQ ID NO: 114);

Please delete the paragraph on page 20, line 24 and replace it with the following paragraph:

Fig. 22 is a figure showing *TipA* gene promoter sequence (SEQ ID NO: 127); and

Please delete the paragraph on page 20, lines 25-26 and replace it with the following paragraph:

Fig. 23 is a figure showing an improvement of the RBS sequence (WTRBS) in *TipA* gene promoter to LG10RBS (SEQ ID NO: 128 and 129).

Please delete the paragraph on page 28, line 23 to page 29, line 5 and replace it with the following paragraph:

Next, amplification by PCR was performed using plasmid pRSET-PIP (Tamura et al., FEBS Lett. 398 101-105 (1996) --- hereinafter abbreviated as PIP) as a template along with primers of SEQ ID Nos. 23 and 24 in the Sequence Listing. The primer of SEQ ID No. 24 in the Sequence Listing is designed so that the termination codon of PIP gene is removed and a 6×His tag (SEQ ID NO: 114) is attached to the C-terminal of the PIP protein for facilitating purification of the protein. The 6×His tag (SEQ ID NO: 114) consists of six contiguous histidine residues and a protein fused with this exhibits high affinity to nickel ion, etc. Therefore, purification becomes easy by the metal chelate chromatography using nickel ion etc. (Crowe et al., Methods Mol. Biol. 31 371-387 (1994)). As a result of double digestion of a DNA fragment of 0.9 kb containing this *PIP* gene by restriction enzymes *Nco*I and *Spe*I and subcloning into *Nco*I and *Spe*I sites of pHN150u, a plasmid containing ORF of *PIP* gene immediately downstream from the promoter sequence of *TipA* gene was created and named pHN151u.

Please delete the paragraph on page 41, lines 8-14 and replace it with the following paragraph:

The transformation of *Rhodococcus erythropolis* JCM3201 strain was carried out in the same manner as in Example 1.2, and PIP was made to express at 30°C and 4°C in the similar manner as in Example 13 using pHN170 (described in Example 7) and pHN171 (described in Example 7), respectively. Here, after adding thiostrepton with a final concentration of 1 µg/ml, cell bodies were collected with time and PIP was purified. The PIP has 6×His tag (SEQ ID NO: 114) linked at C-terminal thereof, and purified using nickel-NTA Superflow (product of Qiagen) following the manufacturer's instructions.

Please delete the paragraph on page 41, lines 15-26 and replace it with the following paragraph:

The purification process was specifically described below, where the procedure of purification was performed at 4°C. The cell bodies which expressed protein (a part for 20 ml culture solution) were collected, and suspended into 1 ml of NT-Buffer (50 mM Tris-HCl (pH 8.0), 100 mM sodium chloride, 1 mM dithiothreitol), and 1 g of glass beads (diameter of 0.105 - 0.125 mm) was added. The cells were destroyed by reciprocally shaking by Fast-prep FP120 (product of SAVANT) with 6 m/second in speed for 20 seconds. After centrifuged in 20,000×g, nickel-NTA Superflow which was beforehand equilibrated with NT-Buffer was added to 700 µl of the supernatant so that the bed volume might be 40 µl. This mixture was subjected to rotation agitation for 1 hour to combine the nickel-NTA Superflow beads to the protein attached with 6×His tag (SEQ ID NO: 114). After washing these beads 4 times by NT-Buffer, the protein was eluted from the protein attached with 6×His tag (SEQ ID NO: 114) by suspending these beads in 120 µl of NTE-Buffer (50 mM Tris-HCl (pH 7.0), 100 mM sodium chloride, 1 mM dithiothreitol, 400 mM imidazole) 3 times.

Please delete the paragraph on page 41, line 27 to page 42, line 17 and replace it with the following paragraph:

The results where 10 µl of the above-mentioned samples was subjected to 12% of SDS polyacrylamide electrophoresis according to an ordinary method are shown in Fig. 16. The transformation of *Rhodococcus erythropolis* JCM3201 strain was carried out by pHN170 (expression from the *TipA* gene promoter: two panels on the left), and pHN171 (expression from the *TipA-LG10* promoter: two panels on the right), and PIP was made to express at 4°C (the upper two panels) and 30°C (lower two panels). After adding thiostrepton with the final concentration of 1 µg/ml, cell bodies were collected with time and purified using nickel-NTA Superflow using the 6×His tag (SEQ ID NO: 114) attached to C-terminal of PIP. The timing for collecting cell bodies is set 0 minute (leftmost lane), 180 minutes (second lane from the left), 420 minutes (third lane from the left), 1080 minutes (fourth lane from the left), 1440 minutes (fifth lane from the left), 1860 minutes (sixth lane from the left), 2520 minutes (seventh lane from the left) and 3060 minutes (eight lane from the left) at 4°C and 0 minute (leftmost lane), 120 minutes (second lane from the left), They are 240 minutes (third lane from the left), 420 minutes (fourth lane from the left), 540 minutes (fifth lane from the left), 720 minutes (sixth lane from the left), 900 minutes (seventh lane from the left) and 1440 minutes (eight lane from the left) at 30°C. In every panel in Fig. 16, the rightmost lane shows the sample purified from cell bodies which continued culturing without

inducing (namely, without adding thiostrepton). At 30°C, expression from the *TipA-LG10* promoter was a little lower compared with the expression from the *TipA* gene promoter, whereas at 4°C, expression from the *TipA-LG10* promoter was conversely higher. In addition, the induction of expression was controlled strictly also in *TipA-LG10* promoter.

Please delete the paragraph on page 43, lines 3-9 and replace it with the following paragraph:

The results where 10 µl of the above-mentioned samples was subjected to 12% of SDS polyacrylamide electrophoresis according to an ordinary method are shown in Fig. 17. The transformation of *Rhodococcus erythropolis* JCM3201 strain was carried out by pHN170 (expression from the *TipA* gene promoter: lanes 1, 3, 5 and 7), and pHN171 (expression from the *TipA-LG10* promoter: lanes 2, 4, 6 and 8), and PIP was made to express at 4°C (lanes 7 and 8), 15°C (lanes 5 and 6), 30°C (lanes 3 and 4) and 32°C (lanes 1 and 2). Purification was performed using nickel-NTA Superflow utilizing the 6×His tag (SEQ ID NO: 114) linked to C-terminal of PIP.

Please delete the paragraph on page 43, line 26 to page 44, line 5 and replace it with the following paragraph:

Amplification by PCR was performed using plasmid pTrc99a-GFP as a template along with primers of SEQ ID Nos. 50 and 51 in the Sequence Listing. Consequently, DNA containing a gene encoding fluorescence green protein derived from *Aequorea victoria* (hereinafter abbreviated as *GFP*) was obtained. Double digestion of this DNA fragment of 0.8 kb was carried out with restriction enzymes *Nco*I and *Sna*BI, and the product was subcloned into *Nco*I and *Sna*BI sites of pTip-NH1 and pTip-LNH1. As a result, plasmids containing *GFP* (having a 6×His tag (SEQ ID NO: 114) linked to N-terminal) gene under control of the *TipA* gene promoter or *TipA-LG10* promoter were created, and the resultant plasmids were named pHN187 and pHN186, respectively.

Please delete the paragraph on page 44, lines 6-13 and replace it with the following paragraph:

Amplification by PCR was performed using plasmid pGEX-2T (Amersham Bioscience Company) as a template along with primers of SEQ ID Nos. 52 and 53 in the Sequence Listing. Consequently, DNA containing a

gene encoding glutathione-S-transferase protein (hereinafter abbreviated as *GST*) was obtained. Double digestion of this DNA fragment of 0.7 kb was carried out with restriction enzymes *NcoI* and *XhoI*, and the product was subcloned into *NcoI* and *XhoI* sites of pTip-NH2 and pTip-LNH2. As a result, plasmids containing *GST* (having a 6×His tag (SEQ ID NO: 114) linked to N-terminal) gene under control of the *TipA* gene promoter or *TipA-LG10* promoter were created, and the resultant plasmids were named pHN282 and pHN283, respectively.

Please delete the paragraph on page 44, lines 23-24 and replace it with the following paragraph:

All of the four above-mentioned proteins have a 6×His tag (SEQ ID NO: 114) and purification was performed in the same manner as Example 16.

Please delete the paragraph on page 44, line 25 to page 45, line 7 and replace it with the following paragraph:

The results where 10 µl of the above-mentioned samples was subjected to 12% of SDS polyacrylamide electrophoresis according to an ordinary method are shown in Fig. 18. The transformation of *Rhodococcus erythropolis* JCM3201 strain was carried out by pHN170 (*PIP* located downstream of the *TipA* gene promoter: lanes 1 and 9), pHN171 (*PIP* located downstream of the *TipA-LG10* promoter: lanes 2 and 10), pHN176 (*AtPIP* located downstream of the *TipA* gene promoter: lanes 3 and 11), pHN177 (*AtPIP* located downstream of the *TipA-LG10* promoter: lanes 4 and 12), pHN187 (*GFP* located downstream of the *TipA* gene promoter: lanes 5 and 13), pHN186 (*GFP* located downstream of the *TipA-LG10* promoter: lanes 6 and 14), pHN282 (*GST* located downstream of the *TipA* gene promoter: lanes 7 and 15) and pHN283 (*GST* located downstream of the *TipA-LG10* promoter: lanes 8 and 16) and the proteins were made to express at 4°C (lanes 9 to 16) and 30°C (lanes 1 to 8). Purification was performed using nickel-NTA Superflow utilizing the 6×His tag (SEQ ID NO: 114) linked to an end of each protein.

Please delete the paragraph on page 55, lines 8-19 and replace it with the following paragraph:

All of these proteins had 6×His tag (SEQ ID NO: 114) linked to the end and were purified as in Example 16. In addition to this, they were purified at this time also from the precipitation (described in Example 16) formed by centrifugation at 20,000×g after cell destruction. The purification process from the precipitation was shown below specifically, and the procedure was conducted at room temperature. The precipitation was suspended into 1 ml DN-Buffer (50 mM Tris-HCl (pH 8.0), 8 M urea) and centrifuged at 20,000×g, and nickel-NTA Superflow which was beforehand equilibrated with DT-Buffer was added to 700 µl of the supernatant so that the bed volume might be 40 µl. This mixture was subjected to rotation agitation for 1 hour to combine the nickel-NTA Superflow beads to the protein attached with 6×His tag (SEQ ID NO: 114). After washing these beads 4 times by NT-Buffer, the protein was eluted from the protein attached with 6×His tag (SEQ ID NO: 114) by suspending these beads in 120 µl of DNE-Buffer (50 mM Tris-HCl (pH 7.0), 8 M urea, 400 mM imidazole) 3 times.

Please delete the paragraph on page 55, line 24 to page 56, line 4 and replace it with the following paragraph:

The purification process from the precipitation was shown below specifically. The cell bodies which expressed a protein were collected and suspended into 1 ml of NT-Buffer. The cells were destroyed by using an ultrasonic generator UD-20 (product of TOMY). The mixture was centrifuged at 20,000×g, and nickel-NTA Superflow which was beforehand equilibrated with NT-Buffer was added to 900 µl of the supernatant so that the bed volume might be 40 µl. This mixture was subjected to rotation agitation for 1 hour to combine the nickel-NTA Superflow beads to the protein attached with 6×His tag (SEQ ID NO: 114). After washing these beads 4 times by NT-Buffer, the protein was eluted from the protein attached with 6×His tag (SEQ ID NO: 114) by suspending these beads in 120 µl of NTE-Buffer 3 times. All of the above-mentioned procedure were conducted at 4°C.

Please delete the paragraph on page 56, lines 8-26 and replace it with the following paragraph:

10 µl of the above-mentioned samples was subjected to 12% SDS polyacrylamide electrophoresis following an ordinary method, and the density of the bands were measured and quantified by densitometer and

the results are shown in Fig. 21. In this figure, the second column from the left shows the name of the protein expressed. The third column from the left shows to which of the N-terminal or C-terminal the 6×His tag (SEQ ID NO: 114) was attached. The fourth column from the left shows presumed molecular weight (kDa) of the full-length protein including signal sequence etc., and the number in parentheses shows presumed molecular weight of the actually expressed portion of the protein. The fifth and ninth columns from the left show the name of the plasmid used when protein was expressed. The sixth, eighth and tenth columns from the left show the mass of the recombinant protein obtained (unit: milligram) per liter and the results purified from the supernatant fraction (Sup) centrifuged at 20,000×g and those purified from the precipitation fraction (Ppt) are separately shown. The symbols + and - in the seventh and eleventh columns from the left show the proliferation rate when each transformant was applied on the agar culture medium containing an expression induction agent (1 µg/ml of thiostrepton in the case of *Rhodococcus erythropolis* and 0.2% L-arabinose in the case of *E. coli*). The transformant proliferated most rapidly is indicated by "+++", and the transformant which did not proliferate at all is indicated by "-". The host used, and the temperature at the time of expression induction are also shown in the topmost part. N.D. (Not Detected) shows that the result was below the detection limit.

Please delete the header for Table 1 on page 57 and replace it with the following header:

Table 1 (6xHis tags disclosed as SEQ ID NO: 114)